ORIGINAL ARTICLE

Factors Associated with *Salmonella* Presence in Environmental Samples and Bulk Tank Milk from US Dairies

J. M. Ruzante[1], J. E. Lombard[2], B. Wagner[2], C. P. Fossler[2], J. S. Karns[3], J. A. S. Van Kessel[3] and I. A. Gardner[4]

[1] Joint Institute for Food Safety and Applied Nutrition, University of Maryland, College Park, MD, USA
[2] Centers for Epidemiology and Animal Health, USDA:APHIS, Fort Collins, CO, USA
[3] Agricultural Research Services, USDA, Beltsville, MD, USA
[4] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA

Impacts

- First national study to investigate factors associated with the presence of *Salmonella* in bulk tank milk and milk filters from US dairy farms.
- National study to examine factors associated with the presence of *Salmonella* in environmental samples of US dairy farms.
- Identified herd practices and characteristics that need further investigation to elucidate their role in reducing *Salmonella* in the environment.

Summary

The objective of this study was to evaluate herd characteristics and management practices associated with presence of *Salmonella* in the farm environment and in bulk tank milk (BTM) in US dairy herds. Herd management data, environmental culture, BTM and in-line milk filter polymerase chain reaction results for *Salmonella* from 260 US dairy herds surveyed during the National Animal Health Monitoring System Dairy 2007 study were analysed. Herd characteristics and management practices were screened by univariate analysis, and selected variables were used to construct a logistic regression model to identify factors associated with the presence of *Salmonella* in environmental samples. To identify factors associated with the occurrence of *Salmonella* in BTM and milk filters, *a priori* selected variables that were related to milking procedures were analysed univariately and a logistic regression model was constructed. The presence of *Salmonella* in the farm environment was associated with location of the operation in the East (OR = 4.8; CI: 1.9–11.6), not using a broadcast manure spreader (OR = 3.2; CI: 1.4–7.5), use of bovine somatotropin (BST) (OR = 2.7; CI: 1.5–5.0) and use of anionic salts (OR = 2.2; CI: 1.2–3.9). In the final multivariable model, herds with fewer than 100 cows were at decreased odds (OR = 0.3; CI: 0.1–0.7) of being culture positive for *Salmonella* as were herds with between 100 and 499 cows (OR = 0.4; CI: 0.2–0.8) compared with herds having 500 or more cows. The presence of culture-positive environmental samples and herd size were significantly associated with *Salmonella* BTM contamination. The herd-level factors identified in this study were in agreement with prior studies but also identified other potential factors that can be targeted in *Salmonella* control programmes.

Introduction

Salmonellosis may cause clinical signs in adult cattle: however, asymptomatic infections are more common in adults as the organism has been frequently isolated from feces of healthy cattle (Wells et al., 2001; Callaway et al., 2005). Salmonellosis is recognized as the second most common bacterial foodborne illness of humans in the United States (Mead et al., 1999). Each year, non-typhoidal *Salmonella* causes an estimated 1.4 million illnesses,

16 000 hospitalizations and 580 deaths at an annual cost of $2.4 billion (Mead et al., 1999; ERS-USDA, 2007). Most non-typhoidal salmonellosis is associated with the consumption of food of animal origin, primarily poultry products; however, milk and dairy products have been frequently implicated in outbreaks (Holmberg et al., 1984; Cody et al., 1999; De Buyser et al., 2001; Mazurek et al., 2004; CDC 2007a). Consumption of fresh fruits and vegetables has been linked with human salmonellosis outbreaks, but the source of the *Salmonella* contamination is often not identified. Cattle, other livestock, and wild animals have the potential to contaminate irrigation pond water, drainage ditches and other environmental locations near produce fields with *Salmonella* or other foodborne pathogens and therefore may represent potential sources of produce contamination (CDC 2007b; Jay et al., 2007; Greene et al., 2008).

Several studies have attempted to identify management practices and herd characteristics that are associated with shedding of *Salmonella* in adult cattle. However, the results have been inconsistent with the exception of herd size which has been repeatedly been associated with identification of *Salmonella* in feces (Vaessen et al., 1998; Kabagambe et al., 2000; Warnick et al., 2001; Huston et al., 2002; Blau et al., 2005). Factors associated with the presence of *Salmonella* in bulk tank milk (BTM) have not previously been identified. Bulk tank contamination is believed to result from faecal contamination attributable to poor milking hygiene rather than intramammary infections with *Salmonella*, which are rare (Van Kessel et al., 2004).

The objectives of the present study were to evaluate factors associated with the presence of *Salmonella* in environmental samples and determine factors associated with its detection in BTM.

Materials and Methods

Surveys

Data were collected during the National Animal Health Monitoring System's (NAHMS) Dairy 2007 study from farms in 17 major dairy states that represented 79.5% of US dairy operations and 82.5% of the dairy cow population. The survey design was a stratified random sample with unequal selection probabilities within each stratum to ensure that large dairy operations were well represented in the sample. Regions and states included in the study were: West: California, Idaho, New Mexico, Texas and Washington; East: Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia and Wisconsin. Operations were categorized into three herd sizes based on the number of adult cows on 1 January 2007 (small = <100 cows, medium = 100–499 and large = 500 or more).

During Phase I of this study, 3554 operations were selected and a general management questionnaire was completed by 2194 dairy operations reporting one or more dairy cows in inventory on 1 January 2007. Operations that completed Phase I with at least 30 dairy cows were eligible for Phase II, and 1077 operations consented to participate. Phase II questionnaires, completed by 582 operations, covered additional areas such as general management, animal health, herd characteristics, handling of manure and waste treatment, milking procedures, biosecurity, and cattle inventory. Details regarding design of the surveys can be found elsewhere (USDA, 2007).

Environmental samples

A convenience subset of 260 dairy operations participating in Phase II took part in the collection of environmental samples to test for *Salmonella*. Composite environmental samples were taken from six different adult-cow areas where manure accumulated on each operation. Recommended locations for sampling included, but were not limited to, common pens or alleyways, manure pit or other manure storage area, holding pens or exit ways from the milking parlor, gutter cleaners, and manure spreaders. For each composite sample, approximately 120 g of manure/slurry were taken from each of six sites within the respective area. For example, for a cow alleyway sample, 120 g of manure were taken from six different locations within the alleyway and combined to form a single composite sample of approximately 720 g. The number of herds to be sampled was based on an expected *Salmonella* herd-level prevalence of 50%, an error margin of 6% in the prevalence estimate, and laboratory capacity. Since another objective in the overall NAHMS study was to estimate prevalence of *Mycobacterium avium* subspecies *paratuberculosis* – the causative agent of Johne's disease, the number of samples and location collected from each operation was based on the guidelines from the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program (USDA-APHIS 2005). Environmental samples were collected from 2 March to 31 August 2007 by Federal and State personnel.

Environmental samples were shipped overnight on ice to the US Department of Agriculture, Agriculture Research Service Bacterial Epidemiology and Antimicrobial Resistance Research Unit (USDA/BEAR) in Athens, GA for culture. Culture of environmental samples was conducted according to the methods previously described (Wells et al., 2001). Briefly, approximately 1 g of faeces from each sample was placed into two culture media (gram-negative Hajna broth and tetrathionate broth) and incubated at 37°C for 24 and 48 h, respectively. One

hundred-microlitre aliquots from each broth were transferred into Rappaport R-10 medium (BD Diagnostics, Sparks, MD, USA) for a secondary enrichment, incubated overnight at 37°C and then streaked onto brilliant green agar with sulfadiazine and xylosine-lysine-tergitol-4 (BD Diagnostics, Sparks, MD, USA) plates. Plates were incubated overnight at 37°C. Up to four colonies with the typical appearance of Salmonella from each plate were inoculated into triple sugar iron and lysine iron agar slants. Slants were incubated overnight at 37°C and *Salmonella* was confirmed using serogroup-specific sera.

Bulk tank milk and in-line milk filters

Bulk tank milk and in-line milk filters samples were collected from 538 operations during the Phase II survey and analyzed by real-time polymerase chain reaction (PCR). Bulk tank milk samples were only taken when at least 70% of the herd's lactating cows were represented in the BTM sample. Additionally, milk filters were collected at the time of bulk tank sampling for evaluation. Filters and BTM were collected from 28 February to 30 August 2007 by the Federal and State personnel and shipped on ice using overnight delivery to the USDA: Agricultural Research Service's Environmental Microbial Safety Laboratory, in Beltsville, MD.

Bulk tank milk and in-line milk filters samples were analyzed for the presence of *Salmonella* by real-time PCR. Briefly, 10 ml of milk were added to 10 ml of 2X tetrathionate broth and incubated overnight at 37°C. Milk filters were cut into pieces and mixed with buffered peptone water in a stomacher bag and pummeled for 2 min. Five millilitres of the liquid of the stomacher bag were added to 5 ml of 2X tetrathionate broth and incubated for 37°C overnight. After incubation, 1.5 ml of the broth was centrifuged (16 000 × *g*) for 2 min in microcentrifuge tubes. The supernatant was discarded, and the DNA was extracted from the pellet biomass using 200 μl of InstageneGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA) following manufacturer's instructions. The DNA preparations were stored at −20°C and analysed for presence of *Salmonella* via PCR for the *invA* gene using the primers described by Rahn et al. (1992) and shown by Malorny et al. (2003) to be effective for the detection of multiple serotypes of *Salmonella enterica* subspecies *enterica*. The PCR reactions were monitored in real time through the addition of EVA-Green dye (Biotium, Inc, Hayward, CA, USA).

Analysis of factors associated with the presence of *Salmonella* in environmental samples

The complete data set included management data and culture results from environmental samples from 251 operations (results from nine operations were not included because of item non-response for independent variables retained in the final model). An operation was considered environmental positive for *Salmonella* when at least one environmental sample was culture-positive for the organism. Operation-level management practices and herd characteristics thought to be related to the presence of *Salmonella* in dairy farms were identified *a priori* based on published studies (Anderson et al., 1997, 2001; Losinger et al., 1997; Vaessen et al., 1998; Kabagambe et al., 2000; Warnick et al., 2001; Wells et al., 2001; Huston et al., 2002; Davis et al., 2003; Callaway et al., 2005; Fossler et al., 2005a,b;) and expert opinion (Randall Anderson, California Department of Food and Agriculture, personal communication). Variables were evaluated for their association with the binary outcome, *Salmonella* contamination of the farm environment, using the crosstab procedure of SUDAAN® (Release 8, 2002; Research Triangle Institute, Research Triangle Park, NC, USA) which accounts for the survey design and allows the inclusions of weights into the model. Variables with the log likelihood chi-square *p*-values <0.05 were selected for the multivariable model. An unweighted forward stepwise selection was used in PROC LOGISTIC in SAS (SAS 9.1; SAS Institute Inc, Cary, NC, USA) to construct the final model. Variables with *p*-value <0.05 were considered significant and included in the final model. The Hosmer and Lemeshow goodness-of-fit test was used to assess the fit of the model.

Analysis of factors associated with presence of *Salmonella* in bulk-tank milk

The complete data set included milking and herd management practices and real-time PCR results from BTM and in-line milk filters from 257 operations from which environmental samples were also taken. Three of the operations from which environmental samples were taken did not have BTM or milk filter samples taken and hence, these operations were excluded from the analysis. Bulk tank milk was considered contaminated with *Salmonella* when BTM, in-line milk filters or both were PCR positive for the organism and the operation was classified as bulk milk positive. *A priori* variables including the environmental testing results were selected and their association with the presence of *Salmonella* in BTM was evaluated using the crosstab procedure of SUDAAN®. Variables with log likelihood chi-square *p*-values <0.05 were selected and a logistic regression model was constructed using an unweighted forward stepwise selection procedure in SAS®. Variables with Wald-*F* statistics with *p*-value <0.05 were considered significant and included in the final model. The fit of the final multivariable model was assessed with the Hosmer and Lemeshow goodness-of-fit statistic.

Table 1. Number (%) of dairy herds by herd size and region (*n* = 260)

| Region | Herd size (number of cows) | | | |
	Small (30–99)	Medium (100–499)	Large (≥500)	Total
West	3 (1.1%)	16 (6.2%)	33 (12.7%)	52 (20.0%)
East	89 (34.2%)	77 (29.6%)	42 (16.2%)	208 (80.0%)
Total	93 (35.3%)	93 (35.8%)	75 (28.9%)	260 (100.0%)

Results

Environmental samples

Salmonella culture results for environmental samples were available for 260 dairy farms. The distribution of these operations by herd size (number of cows) and region is presented in Table 1. One hundred and fourteen (43.8%) had at least one environmental sample that was culture positive for *Salmonella* (Table 2).

Bulk tank milk and in-line milk filters

Real-time PCR results for BTM and milk filters were available for 257 of the 260 dairy farms where environmental samples were collected, and 89 (34.6%) were PCR-positive for *Salmonella* in either BTM, milk filter or both. Of the 89 PCR positive samples, 26 were positive in BTM and 82 were positive using milk filters. Samples from 19 and 168 operations were PCR-positive and PCR-negative for *Salmonella* in both milk and filters respectively.

Of the 257 operations that had both milk/milk filters sampled and environmental samples collected and tested for *Salmonella*, 111 were negative (43.2%) and 55 were positive (21.4%) for both milk/milk filter and environmental sample. There were 89 BTM PCR-positive operations and 55 of these operations had at least one environmental positive.

Table 2. Distribution of *Salmonella* spp.-positive environmental samples in 260 US dairies

No. positive environmental samples	Percent of herds (*n*)
0	56.2 (146)
1	12.7 (33)
2	4.6 (12)
3	3.1 (8)
4	4.6 (12)
5	6.5 (17)
6	12.3 (32)

Analysis of factors associated with the presence of *Salmonella* in environmental samples

Ninety-two general dairy management practices were chosen *a priori* for investigating potential association with *Salmonella* contamination of the farm environment. Thirty-five were significant at $P < 0.05$ in the univariate analysis and were selected for the multivariable logistic regression model. Season was also considered in the univariate analysis, but there was no significant difference between spring (March–May) and summer (June–August) ($P = 0.24$). Five variables remained in the final model at $P < 0.05$. No two-way interactions were significant.

The final model indicated that the presence of *Salmonella* in environmental samples was associated with location of the dairy operation in the East region, not using a broadcast manure spreader, herd size of 500 or more milk cows, the use of bovine somatotropin (BST) or anionic-salts (Table 3). The Hosmer and Lemeshow goodness-of-fit test indicated excellent model fit ($P = 0.998$).

Analysis of factors associated with presence of *Salmonella* in bulk tank milk and milk filters

Forty milking practices and herd management characteristics were screened for univariate association with the presence of *Salmonella* in bulk-tank milk and milk filters. Seven variables (herd size, environmental-positive for *Salmonella*, who milks the majority of cows on the operation, isolation of *Mycoplasma* from bulk-tank milk during the study, latex or nitrile gloves worn by workers during milking, use of automatic takeoffs during milking, and any training of milkers) were significantly associated with BTM contamination of *Salmonella* in the univariate analysis (Table 4) and were offered to the multivariable model. Season was also considered in the univariate analysis; however, there was no significant difference between spring and summer ($P = 0.15$).

The final multivariable model included two of the seven variables. The presence of at least one culture-positive environmental sample and herd size were significantly associated with *Salmonella* BTM contamination (Table 5). In addition, we found an interaction between herd size and environmental sample results that was significant ($P = 0.03$).

The interaction showed that herd size was not influential in predicting *Salmonella* in BTM or milk filters when *Salmonella* was isolated from the environment. However, in herds without positive environmental samples, the odds of finding *Salmonella* in BTM or milk filters increased with herd size. Compared with the reference category of no evidence of *Salmonella* environmental con-

Table 3. Final multivariable model of factors associated with the presence of *Salmonella* in environmental samples in 251 US dairies

Variable	Variable categories	Coefficients	P-value	Odds ratio	95% confidence interval
Intercept	–	−1.29	<0.001		
Use anionic salts	Yes	0.39	0.009	2.17	1.21–3.89
	No	Reference			
Use BST	Yes	0.99	0.002	2.68	1.46–4.95
	No	Reference			
Use broadcast manure spreader	No	1.16	0.008	3.20	1.36–7.53
	Yes	Reference			
Region	East	1.56	<0.001	4.76	1.95–11.61
	West	Reference			
Herd size (adult cows)	30–99	−1.28	0.004	0.28	0.12–0.66
	100–499	−0.97	0.014	0.38	0.18–0.82
	≥ 500	Reference			

Table 4. Univariate analysis of factors associated with *Salmonella*-positive BTM or milk filters in 257 US dairies

Variable	Variable categories	Percent of herds	Percent of BTM + herds	P-value
Salmonella environmental contamination	Positive	43.6	38.2	<0.001
	Negative	56.4	61.8	
Herd size (milk cows)	30–99	35.0	28.1	0.012
	100–499	36.2	31.5	
	≥500	28.8	40.4	
Mycoplasma isolated from bulk tank milk during the study	Yes	3.5	5.6	0.026
	No	96.5	94.4	
Who milked the majority of cows on the operation	Owner	35.8	24.7	0.023
	Family member	9.3	9.0	
	Hired labor	54.9	66.3	
Workers wear latex or nitrile gloves during milking	Yes	73.2	82.0	0.026
	No	26.8	18.0	
Operation uses automatic takeoffs during milking	Yes	69.4	77.5	0.048
	No	30.6	22.5	
Milker training is performed	Yes	73.0	80.7	0.050
	No	27.0	19.3	

Table 5. Final multivariable model of factors associated with the presence of *Salmonella* in BTM or milk filters in 257 US dairies

Variable	Variable categories	Coefficients	P-value
Intercept	–	−1.61	<0.001
Salmonella environmental contamination	Positive	1.58	<0.001
	Negative	Reference	
Herd size (adult cows)	≥500	1.54	<0.001
	30–499	Reference	
Env. contamination*herd size	–	−1.56	0.008

tamination in small herds (30–499 cows), odds ratios were virtually identical for *Salmonella* environmental contamination in small herds, *Salmonella* environmental contamination in large herds (≥500 cows), and no *Salmonella* environmental contamination in large herds (OR = 4.85,

4.75 and 4.66 respectively). The fit of the final model was adequate (Hosmer and Lemeshow goodness-of-fit test: $P = 0.41$).

Discussion

The objective of this study was to identify herd-level factors associated with the presence of *Salmonella* in environmental samples and BTM/milk filters from US dairy farms. Although a convenience sample, the 260 herds that were included in this study of factors associated with the presence of *Salmonella* in environmental samples and BTM are believed to reflect the diversity of regions and herd sizes of the US dairy population. The main strengths of the study were the national scope and the detailed and comprehensive questionnaires which captured extensive herd information. In addition, all samples were analysed

using standardized methods within the Agriculture Research Services laboratories of USDA, which allowed for better comparison among farms.

The two previous national studies, NAHMS Dairy 1996 and NAHMS Dairy 2002, estimated the faecal prevalence of *Salmonella* in US dairy herds by sampling individual animals in 91 and 97 dairy herds respectively. NAHMS Dairy 1996 has also identified factors associated with shedding of *Salmonella* in adult cattle and the 2002 study used real-time PCR and culture methods to determine the prevalence of this pathogen in BTM. This is the first national dairy study that has utilized environmental samples, bulk-tank milk and milk filters to identify *Salmonella*-infected herds and their association with management practices and herd characteristics. This study also surveyed a much larger number of operations than previous NAHMS dairy studies.

Environmental sampling has previously been found to be useful for identifying *Salmonella*-infected dairy operations (Warnick et al., 2003) and has been shown to be a cost-effective method for the identification of *Mycobacterium avium* subsp. *paratuberculosis* when compared with other diagnostic methods including individual faecal culture (Raizman et al., 2004; Berghaus et al., 2006; Tavornpanich et al., 2008). One disadvantage of environmental sampling, especially for *Salmonella* that has a broad range of host species, is that it is not possible to establish with certainty that the animals in the herd are in fact infected with the pathogen. Nevertheless, in this study the environmental samples collected (with exception of manure storage systems), were primarily faecal material that had been deposited within 12 h prior to sampling, minimizing the chances that the *Salmonella* isolated were from host species other than cows in the herd.

In the present study, there was a significant association between geographical region and environmental contamination with *Salmonella*. In the multivariable model, dairies in the East were more likely to be positive for *Salmonella* than dairies in the West. Region was also a significant factor associated with faecal shedding of *Salmonella* in the 91 herds evaluated during NAHMS Dairy 1996 (Kabagambe et al., 2000). In another study limited to the Midwest and New York, Midwest farms were more likely to be *Salmonella*-positive than farms in New York (Fossler et al., 2005a). It is difficult to compare regional results from NAHMS Dairy 1996 and other studies with the NAHMS Dairy 2007 results because different states and regional groupings were used and because it is possible that survivability of *Salmonella* in the environment in different regions of the country may have affected the results. It is apparent that regional differences in presence of *Salmonella* can exist, but there is a lack of current evidence indicating that these regional differences appear consistently over time.

Other studies have reported higher *Salmonella* faecal prevalence during summer as opposed to winter (Edrington et al., 2004; Fossler et al., 2005a,b). In this study, season was not associated with the presence of *Salmonella* in environmental samples or in BTM and milk filters in both univariate analyses. However, the comparison was made only between two seasons of the year (spring and summer) which could explain the failure to detect a significant association.

The association with herd size was expected as other authors have repeatedly found strong associations between large herd sizes and detection of *Salmonella* shedding (Vaessen et al., 1998; Kabagambe et al., 2000; Warnick et al., 2001; Huston et al., 2002; Blau et al., 2005). In this study, smaller herd sizes, 30–99 and 100–499 milking cows, had lower odds of being *Salmonella* positive when compared to herds with 500 or more milk cows. More animal-dense operations and certain management practices used by larger herds have been reported as factors associated with *Salmonella* shedding (Warnick et al., 2001; Wells et al., 2001).

Herds that did not use a broadcast manure spreader were 3.2 times more likely to have at least one culture-positive environmental sample. These results agree with previous work that found that disposal of manure by irrigation or application of slurry (by surface or subsurface) was associated with a 2.1 times greater odds for *Salmonella* shedding by cows when compared with disposal of manure by a broadcast spreader only (Fossler et al., 2005b). Other manure management practices such as spreading of slurry on pastures (Jack and Hepper, 1969) and use of lagoon water for irrigation of crops (Anderson et al., 2001), have previously been identified as factors associated with shedding of *Salmonella*.

The association of anionic salts use with the presence of *Salmonella* in the farm environment was unexpected. Compared with herds that did not use anionic salts, herds that did use them (46%) were at 2.2 greater odds of being *Salmonella*-positive. Anionic salts are commonly used to prevent clinical hypocalcemia (milk fever) and a consequence of this treatment is the reduction of blood and urine pH. To our knowledge, the pH of feces during use of anionic salts has not been evaluated, but the pH of the blood and ruminal fluid decreases when animals are receiving such diets (Tucker et al., 1992). The association between the use of anionic salts and the presence of *Salmonella* in environmental samples may arise from a dietary imbalance that causes slight changes in physiological pH. This physiological change could affect the viability of the normal microflora and provide better conditions for *Salmonella* to grow and/or be isolated in the faecal sam-

ple. Imbalance of cation-anion diets has previously been implicated as a potential cause of an outbreak of clinical salmonellosis in a dairy farm in California (Randall Anderson, California Department of Food and Agriculture, personal communication).

The efficacy of *Salmonella* or coliform vaccines to reduce shedding of *Salmonella* is frequently questioned. In this study, the use of both vaccines above and *Mycoplasma* vaccines, were univariately associated with the presence of *Salmonella* in the environment, but the variable was not retained in the final multivariable model. Previous studies have found that feeding whole cottonseed meal (Anderson et al., 1997; Losinger et al., 1997) and access to pasture (Vaessen et al., 1998; Fossler et al., 2005b) could be a risk factor for *Salmonella* in dairy herds; however, in the present study both variables were identified as significant in the univariate analysis but were not retained in the multivariable model.

The biological significance of the use of BST and higher odds for presence of *Salmonella* in the environment are unclear and may represent management practices that were not captured in this study but are related to its use. Another possible explanation could be the potential effect that the use of BST has in the overall health and performance of dairy cattle which could in potentially increase the risk of *Salmonella* infection. In a meta-analysis review on the effects of BST on dairy cattle health, reproductive performance and culling, cows treated with BST had increased milk production, lower body scores, and were at greater risk for clinical mastitis and for developing clinical signs of lameness when compared with untreated cows (Dohoo et al., 2003a; b). This same study pointed to an apparent increase in the risk of culling for multiparous cows (Dohoo et al., 2003b). A study from Wells et al. (2001) showed that cows in cull markets had higher prevalence of *Salmonella* than cows on dairy farms. Therefore, the fact that cull cows could be at higher risk of shedding *Salmonella* and also have higher chances of being treated with BST could help explain this association; however this is a hypothesis that would need to be evaluated by other studies.

In the present study, 34.6% (89/257) of the operations were PCR positive for *Salmonella* in BTM or in milk filters. Almost four times the number of *Salmonella* infected herds were detected using PCR methods on milk filters alone ($n = 82$) compared to BTM PCR alone ($n = 26$). Nineteen of the 89 positive herds were detected by both BTM and milk filters PCR methods. In a prior study (Fossler et al., 2005a), in-line milk filters were about three times more likely to be *Salmonella* positive than BTM. Therefore, milk filters might be a sensitive indicator of BTM contamination as they gather and concentrate material from different sources into a single channel and the entrapped material may aid the attachment of bacteria to the filter, increasing the chances of detection (Hassan et al., 2000; Van Kessel et al., 2008).

Few studies have attempted to identify factors associated with the presence of *Salmonella* in BTM or milk filters. To our knowledge, this is the first study to evaluate a large number of herd management variables and milking procedures for association with the presence of *Salmonella* in BTM or milk filters. In the present study, only *Salmonella* isolation in environmental samples, herd size and their interaction were retained in the final milk model. *Salmonella* was more likely to be present in BTM or milk filters when at least one environmental sample was positive. In herds with positive environmental samples, the odds of finding *Salmonella* in BTM or milk filters was not affected by herd size; however, in herds without positive environmental samples, the odds of finding *Salmonella* in BTM or milk filters increased with herd size.

The presence of *Salmonella* in milk is believed to be mainly attributable to faecal contamination because of poor milking hygiene and contamination of milking equipment rather than direct shedding of the organism in milk (Jayarao et al., 2006). In this study, milking hygiene practices and herd management were not retained in the final model; however, five variables: who milks the majority of cows on the operation, whether *Mycoplasma* was isolated from BTM during the study, if workers wear latex or nitrile gloves during milking, if the operation uses automatic takeoffs during milking, and if any milker training was performed were found to be significantly associated with *Salmonella* in the univariate analysis and their association with the presence of *Salmonella* in bulk-talk milk should not be ruled out and deserves further investigation.

This is the first reported study that has thoroughly investigated diverse factors associated with the presence of *Salmonella* in environmental samples and bulk-tank milk/milk filters in US dairy farms. Two hundred and sixty operations of different herd sizes from the main dairy states were evaluated. Our findings were generally consistent with prior risk-factor studies of *Salmonella* in dairy herds. In addition, we identified new practices and characteristics that warrant further investigation as potential control options for *Salmonella* in dairy herds.

Acknowledgements

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